

**CLAIMS**

1. A method for detecting a nucleic acid molecule having a particular nucleotide sequence immobilized to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is for a time and under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence with greater efficiency and/or specificity compared to the nucleic acid primer which contains a mis-match and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.
2. A method according to Claim 1 wherein a solution phase primer is allele specific and is capable of extending from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized.
3. A method for detecting a nucleic acid molecule having a particular nucleotide sequence immobilized to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said

at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.

4. A method according to Claim 1 or 2 or 3 wherein the competitive priming step occurs after amplification with a generic primer.

5. A method for detecting a nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using at least two solution phase primers and anchoring said amplified nucleic acid molecule to the solid support, contacting said anchored nucleic acid molecule with at least two other solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence with greater efficiency and/or specificity compared to the nucleic acid primer which contains a mis-match and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule.

6. A method according to Claim 5 wherein the solution phase primer is allele specific and is capable of extending from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized.

7. A method for detecting a nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using at least two solution phase primers and anchoring said amplified nucleic acid molecule to the solid support, contacting said anchored nucleic acid molecule with at least two other solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule.

8. A method for detecting a nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using at least two solution phase primers having a high  $T_m$  and wherein the amplification conditions are such that said solution phase primers are active and anchoring said amplified nucleic acid molecule to the solid

support, contacting said anchored nucleic acid molecule with at least two other solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said latter two primers have a  $T_m$  lower than the temperature used in said first amplification such that during the first amplification, the second set of primers is inactive but during said second amplification, said second set of primers is active, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule..

9. A method for detecting a nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using a solution phase primer having a high  $T_m$  and wherein the amplification conditions are such that said solution phase primers are active and anchoring said amplified nucleic acid molecule to the solid support, contacting said anchored nucleic acid molecule with at least two other solution phase nucleic acid primers each having a low  $T_m$  wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said latter two primers have a  $T_m$  lower than the temperature used in said first amplification such that during the first amplification,

the second set of primers is inactive but during said second amplification, said second set of primers is active, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule.

10. A method according to Claim 8 or 9 wherein the immobilized primer has a high  $T_m$ .

11. A method according to Claim 8 or 9 wherein the immobilized primer has a low  $T_m$ .

12. A solid support comprising an array of immobilized primers wherein each of the primers may comprise the identical nucleotide sequence or one or more may differ from each other by at least one nucleotide and wherein the array is used to detect a nucleic acid molecule having a particular nucleotide sequence immobilized to a solid support via hybridization of said nucleic acid molecule to said one or more primers anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence with greater efficiency and/or specificity compared to the nucleic acid primer which contains a mis-match and then detecting the relative presence of

a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.

13. A solid support according to Claim 12 wherein a solution phase primer is allele specific and is capable of extending from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized.

14. A solid support comprising an array of immobilized primers wherein each of the primers may comprise the identical nucleotide sequence or one or more may differ from each other by at least one nucleotide and wherein the array is used to detect a nucleic acid molecule having a particular nucleotide sequence immobilized to a solid support *via* hybridization of said nucleic acid molecule to said one or more primers anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence but at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.

15. A nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using at least two solution phase primers having a high  $T_m$  and wherein the amplification conditions are such that said solution phase primers are active and anchoring said amplified nucleic acid molecule to the solid support, contacting said anchored nucleic acid molecule with at least two other solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said latter two primers have a  $T_m$  lower than the temperature used in said first amplification such that during the first amplification, the second set of primers is inactive but during said second amplification, said second set of primers is active, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule.

16. A nucleic acid molecule having a particular nucleotide sequence anchored to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising subjecting said nucleic acid molecule to amplification using a solution phase primer having a high  $T_m$  at least two solution phase primers having a high  $T_m$  and wherein the amplification conditions are such that said solution phase primers are active and anchoring said amplified nucleic acid molecule to the solid support, contacting said anchored nucleic acid molecule with at least two other

solution phase nucleic acid primers each having a low  $T_m$  wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the anchored nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said latter two primers have a  $T_m$  lower than the temperature used in said first amplification such that during the first amplification, the second set of primers is inactive but during said second amplification, said second set of primers is active, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the anchored nucleic acid molecule.

17. A method for discriminating between nucleotide repeat number polymorphism on a nucleic acid molecule immobilized to a solid support via hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is for a time and under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence with greater efficiency and/or specificity compared to the nucleic acid primer which contains a mis-match and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to

the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.

18. A method according to Claim 17 wherein a solution phase primer is allele specific and is capable of extending from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized.

19. A method for discriminating between nucleotide repeat number polymorphism on a nucleic acid molecule immobilized to a solid support via hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primer which is complementary to the target sequence to hybridize to said target sequence and to extend from its 3' terminus to form an extension product complementary to the strand to which the primer has hybridized with greater efficiency and/or specificity compared to a mis-matched primer and then detecting the relative presence of a signal wherein the relative presence of said signal is indicative of which primer has hybridized to the target sequence depending on which primer has been labelled and the relative presence of a signal is indicative of the identity of the immobilized nucleic acid molecule.

20. A method according to Claim 17 or 18 or 19 wherein the nucleotide repeat number polymorphism is microsatellite DNA.

21. A method according to Claim 17 or 18 or 19 or 20 to detect a neurodegenerative disease.

22. A method according to Claim 21 wherein the neurodegenerative disease is Huntington's disease.

23. A method for detecting a nucleotide repeat number polymorphism in a target nucleic acid molecule, said method comprising subjecting said target nucleic acid molecule to interrogation by a pair of immobilized primers wherein said primers are immobilized in separate reaction vessels or separate spot arrays and wherein one primer is capable of priming a particular nucleotide length polymorphism whereas the other primer is unable to induce priming of said nucleotide length polymorphism wherein at least one primer is labelled with a reporter molecule capable of providing an identifiable signal wherein the relative presence or absence of said signal is indicative of the presence or absence of said nucleotide length polymorphism.

24. A method according to Claim 23 wherein the nucleotide repeat number polymorphism is microsatellite DNA.

25. A method according to 23 to detect a neurodegenerative disease.

26. A method according to Claim 23 wherein the neurodegenerative disease is Huntington's disease.

27. A method for quantitating a nucleic acid molecule immobilized to a solid support *via* hybridization of said nucleic acid molecule to a primer anchored to said solid support, said method comprising contacting said immobilized nucleic acid molecule with at least two solution phase nucleic acid primers wherein the nucleotide sequence of at least one of the primers is complementary to a target nucleotide sequence within or on the immobilized nucleic acid molecule and wherein the nucleotide sequence of at least another primer differs from said target nucleotide sequence by at least one nucleotide mis-match and wherein at least one of said at least two primers is labelled with a reporter molecule capable of providing an identifiable signal, wherein said contact is under conditions sufficient for the nucleic acid primers to amplify said target sequence to completion of an

amplification reaction wherein the amount of target nucleic acid molecule is proportional to the number of amplification cycles such that the amount of target nucleic acid molecule is determined from the ratio of incorporation of complementary and mis-match primers at the end of the amplification reaction.